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Atrial Fibrillation-Induced Gap Junctional Remodeling

With great interest we have read the study by Polontchouk et al. (1) on changes in the distribution of gap junctions—clusters of intercellular channels that allow action potentials to propagate from one cardiomyocyte to another—during atrial fibrillation (AF). In atrial tissue from chronic AF patients, the researchers observed a 2.7-fold increase in connexin40 (Cx40) gap junction protein while levels of Cx43 had not changed. For both connexins, a partial redistribution from polar to lateral cell membranes was observed. Possible biophysical implications were tested by determination of changes in anisotropy—ratio of longitudinal (V_L) and transverse (V_T) conduction velocities—in a rat model of pacing-induced AF (24 h at 10 Hz) showing similar changes in Cx43 distribution.

First, we would like to comment on the immunohistochemical analyses performed. The antibody against Cx40 was said to recognize a 40-kDa protein in adult rat ventricular cardiomyocytes. Because these cells do not express Cx40, this suggests cross-reactivity with Cx43. Immunohistochemical staining of human tissue is often hampered by high levels of autofluorescence of extracellular matrix components (elastin). In this context only some of the blue arrows in their Figure 3 (1) point at lateral connexin labeling, suggesting "connexin lateralization" to be smaller than indicated.

Second, the investigators speculate that the observed connexin redistribution may contribute to the formation of an arrhythmogenic substrate for AF, based on their finding in the rat model of a ~40-fold increase in lateral Cx43 staining from 1.5% to 61% in parallel to a change in anisotropy from 3.6 ± 0.5 to 2.4 ± 0.1 (from text; for the same set of data, their Figure 6 shows 3.8 ± 0.8 and 1.9 ± 0.2, respectively). However, lateral Cx43 staining in rat atrial tissue kept at sinus rate for 24 h increased 6-fold (from 1.5% to 9.0%; as shown in their Figure 5) without any change in anisotropy. Both from this observation and from model studies on cardiac action potential conduction (e.g., by Kléber [2], Spach et al. [3], and Jongsma and Wilders [4]) we believe the functional significance of the much smaller increase in connexin staining in human atrial tissue (from 5% to 16%, and from 9% to 22% for Cx43 and Cx40, respectively) might be minimal.

In the goat we found AF-induced inhomogeneities in the atrial distribution pattern of Cx40 consisting of small (0.15 to 0.6 mm diameter) areas of low to almost zero density (5). We hypothesize that this phenomenon, rather then "lateralization" of gap junctions, forms a likely basis for the generation of micro-heterogeneity (or micro-dispersion) in conduction velocity and might support microreentry, which could lead to sustained AF.

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REPLY

In our study (1) we have shown changes in the expression and localization of the gap junction proteins connexin40 (Cx40) and connexin43 (Cx43) in patients suffering from chronic atrial fibrillation (AF). We found increased Cx40 and unchanged Cx43 expression. Moreover, we observed lateralizations of both connexins that were no longer confined to the cellular poles. In a rat model of pacing-induced AF, lateralization was also found along with a change in anisotropy.

Regarding the communication from van der Velden and colleagues, there is pobably some misunderstanding: It is stated clearly in our report that we produced a polyclonal antibody against a polypeptide comprising amino acids 231–330 of rat Cx40. There is no putative amino acid sequence homologous to Cx43.

This antibody did not show crossreactivity with other connexins. This was tested in Western blots from HeLa cells transfected with either Cx43 or Cx40. Immunocytochemistry demonstrated that Cx40 staining differs from Cx43 staining. In rat and mice, we did not see any Cx40 staining in the smooth muscle cells (SMC) of aortae, whereas Cx43 antibodies recognized Cx43 in SMC. The specificity of the antibody has also been shown in rat kidney (2). When freshly isolated ventricular cardiomyocytes from two-month-old rats were cultured, redifferentiation was found with differential time-dependent changes in connexin expression and phosphorylation and no crossreactivity between Cx40 and Cx43.

Regarding the immunostainings, there is autofluorescence from connective tissue. However, this is typical for this kind of human tissue. It is difficult to differentiate between connective tissue and specific staining (especially in the reproductions), but it is possible because the connective tissue shows typical thin, *curly*, fluorescent *lines*. Moreover, there is yellowish fluorescence from lipofuscine, which also disturbs. Thus, although we agree that it is difficult to evaluate, we have seen enhanced presence of specific immunofluorescence at the lateral sides of the cells in AF patients.